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Karen Zieden
Name

July 30, 2004
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: J. Clark & C. Denning

Filing Date: June 13, 2000

Serial No: 09/593,316

Docket: 730/002

Title: ANIMAL TISSUE FOR XENOTRANSPLANTATION

Art Unit: 1632

Examiner: Qian J. Li, Ph.D.

RESPONSE TO OFFICE ACTION
UNDER 37 CFR § 1.111

Commissioner for Patents
Alexandria VA 22313

Dear Sir,

This paper is responsive to the most recent Office Action on the merits, dated January 30, 2004, for which a response is due April 30, 2004. Accompanying this Response is a Request for a three month extension of time, setting the due date to July 30, 2004. Accordingly, this response is timely filed.

No amendments are made to the claims or the disclosure. Please enter the following remarks.

Status of the application:

The claims under examination were rejected in three Office Actions, subsequent to which applicant appealed to the Board of Patent Appeals and Interferences. The Appeal Brief was filed on September 4, 2003.

Prosecution was subsequently reopened by the Examiner, and a new Office Action was mailed January 30, 2004.

Claims 1-7, 13-17, 22, and 27-37 are pending in this application. Claims 1-6 and 33-37 were previously rejected. The Office Action indicates that Claims 13-16 have now been rejoined back into the group under examination, for which applicant is grateful. In fact, claims 13-15 were previously rejoined on November 23, 2001 (Paper No. 7). Applicant gratefully acknowledges confirmation that claims 13-15 are included in the group under examination, and that claim 16 has now been rejoined as well. Accordingly, claims 1-6, 13-16, and 33-37 are currently under examination.

Certain claims stand newly rejected under 35 USC § 101. The rejection under the written description requirement of § 112 ¶ 1 has been withdrawn, for which applicant is grateful.

Applicant requests reconsideration and allowance of the application.

Rejection under 35 USC § 101

Claims 1-6 and 33-37 stand rejected under § 101 as claiming subject matter without a credible asserted or a well-established utility.

The Office Action indicates that the claimed invention has no utility with respect to homozygous α1,3GT knockout animals and tissues, because the specification does not enable the making of homozygous α1,3GT knockouts. The Examiner is respectfully reminded that the utility requirement of § 101 does not depend on whether the specification enables the claimed invention. Enablement issues should be raised under 35 USC § 112 ¶ 1. As indicated throughout the specification, ovine tissue devoid of antibody-detectable Galα(1,3)Gal determinants are useful and under development in a number of laboratories for use in xenotransplantation.

The Office Action indicates that the claimed invention has no utility with respect to heterozygous α1,3GT knockout animals and tissues, because there is no phenotypic difference from normal ovine animals and tissues. However, the Office Action provides no rationale why heterozygous animals would be phenotypically identical to normal animals. In fact, there would probably be a gene dosing effect: with only one allele instead of two, the number of α1,3GT molecules in the cell may be half of what is normal, leading to a *lower density* of Galα(1,3)Gal

determinants on the cell surface. Just decreasing the density of Galα(1,3)Gal antigen improves xenograft acceptance (Costa et al., FASEB J. 17:109, 2003). The specification refers to Galα(1,3)Gal density on page 42, and references U.S. Patent No. 5,849,991.

Second, the specification teaches on page 41, lines 14-17 that heterozygous α1,3GT knockout animals have utility for making homozygous knockout animals by cross-breeding. The use of heterozygous knockout animals to breed a homozygous knockout animal is covered in Claim 13, which has not been rejected under § 101. Crossbreeding of heterozygous knockouts has been used successfully to generate α1,3GT knockouts in mice (U.S. Patent No. 5,849,991).

Third, the specification teaches on page 41, lines 17-20 that tissue from heterozygous α1,3GT knockout animals (both birthed animals and fetuses) have utility for targeting the second allele, thereby obtaining homozygous α1,3GT knockout cells. This in turn can be used for nuclear transfer for production of homozygous knockout animals. Second allele targeting and recloning has been used successfully to generate α1,3GT knockouts in pigs (Phelps et al., Science 299, 411-414, 2003; Kolber-Simonds et al., Proc. Natl. Acad. Sci. USA 101:7335, 2004).

Thus, there are several asserted and credible utilities of α1,3GT heterozygous cells and animals that meet the requirements of § 101. Withdrawal of this rejection is requested.

Rejection under 35 USC § 112 ¶ 1:

Claims 1-6, 13-16, and 33-37 stand rejected under the enablement requirement of § 112 ¶ 1. This is contrary to the Office Action issued on this application on November 23, 2001, which says that the specification is enabled for homozygous inactivation of the α1,3GT gene in Finn Dorset sheep. The present Office Action asserts that the specification is not enabling for any claimed subject matter. The following reasons are given:

1. The specification provides no working example of a homozygous α1,3GT knockout cell, or a heterozygous α1,3GT knockout animal;
2. Cloning by nuclear transfer is unreliable, and not just a matter of routine experimentation;
3. Where a transgene is inserted into a cell has an uncertain effect on phenotype, because of the site of integration, transgene copy number, insertional mutations; and lesions in the background;
4. Contrary to the pig, knocking out the α1,3GT gene kills sheep fetuses.

Applicant respectfully disagrees with all these reasons.

1. Working Example

The enablement requirement of 35 USC § 112 ¶ 1 does not require that the specification contain a working example.

> It is well established in the law that a specification can adequately describe the manner and process of making an embodiment of an invention, whether or not it has actually been conducted. Use of prophetic examples does not make a patent non-enabling. The burden is on the person challenging the patent to show . . . that the prophetic examples together with other parts of the specification are not enabling. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 224 USPQ 409 (Fed. Cir. 1984).

The burden is on the Patent Office to show that the specification in combination with general methodology known in the art at the time of filing still does not provide enough information for the skilled reader to make the invention without undue experimentation.

Methods of inactivating genes by homologous recombination, and methods of nuclear transfer to make genetically modified animals are both well known in the art. This application discloses the sequence of the sheep α1,3GT gene needed to create α1,3GT knockouts, as has been done for other species. The Office has not explained exactly what information is missing that is requied to carry out the invention.

2. The Art of Nuclear Transfer

Office Action cites an articles by Donovan and Gearhart (Nature 414:92, 2001) and Simerly et al. (Science 300:297, 2003) as confirming that cloning is difficult or impossible. However, the topic of both these papers is the *cloning of humans and other higher primates*. Unless things have changed recently, it is applicant's understanding that sheep are not considered members of the primate family.

The position taken in the Office Action is contrary to the position of the U.S. Patent & Trademark Office with respect to a number of issued cases in the field of nuclear transfer: see, for example, U.S. Patent Nos. 6,147,276; 6,252,133; and 6,525,243 (Campbell & Wilmut, Roslin Institute); U.S. Patent Nos. 5,945,577; 6,215,041; 6,235,969; and 6,235,970 (Stice et al., U. Mass.); U.S. Patent Nos. 6,258,998 and 6,700,037 (Damiani et al., Infigen); and U.S. Patent Nos. 6,011,197; 6,395,958; and 6,603,059 (Strelchenko et al., Infigen).

These patents claim or pertain to production of animals by nuclear cloning. The patents by Campbell and Wilmut are exemplified by the cloning of *Dolly the Sheep*. The patents are all

presumed enabled under 35 USC § 282. Surely the Examiner is not suggesting that the Office has changed its mind, and has decided to take all of these patents back.

At the time of filing of this application, cloning of large animals by nuclear transfer was a well established technique. The frequency of successful cloning events is not an issue, as long as it can be done without undue experimentation. Undue experimentation is not required to carry out the inventions in U.S. Patent Nos. 6,147,276; 6,252,133; 6,525,243; 5,945,577; 6,215,041; 6,235,969; 6,235,970; 6,258,998; 6,700,037; 6,011,197; 6,395,958; and 6,603,059 So why should there be undue experimentation for cloning by nuclear transfer in the present context?

There is no evidence of record to indicate that nuclear transfer cannot be done by established methods when the donor cell has been genetically altered.

In fact, there have been a number of successful reports of cloning genetically altered animals according to the Campbell & Wilmut method. For example, the following papers were referred to in the Appeal Brief:

1. Uchida et al. (Transgenic Research 10:577, 2001) report the production of transgenic miniature pigs by pronuclear microinjection. The Huntington gene cloned from miniature pig, was linked to rat enolase promoter, and injected into pronucleus of fertilized eggs. Several of the offspring were determined to have the transgene by PCR and Southern analysis.
2. Bondoli et al. (Molec. Repro. Dev. 60:189, 2001) report cloned pigs generated from cultured skin fibroblasts derived from a boar with an H-transferase transgene. Two healthy piglets resulted from nuclear transfer by fusion of fibroblasts that had been extensively cultured with enucleated oocytes.
3. Lai et al. (Molec. Repro. Dev. 62:300, 2002) report a transgenic pig expressing green fluorescence protein. Fetal-derived fibroblast cells were transduced with the GFP gene, and then cloned into porcine oocytes. A healthy transgenic piglet was obtained that expressed GFP.
4. McCreath et al. (Nature 405:1004, 2000) report transgenic sheep made by nuclear transfer from fibroblast donors in which different transgenes were targeted into the alpha1(I) procollagen locus.

5. Lai et al. (Science 295:1089, 2002) report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pigs were produced by nuclear transfer, using clonal fetal fibroblast cell lines as nuclear donors.
6. Dai et al. (Nature Biotech 20:251, 2002) also report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pig α1,3GT gene was disrupted in both male and female porcine primary fetal fibroblasts, which were then used for nuclear transfer. Six clonal fetal piglets were obtained, of which five were normal weight and apparently healthy. Southern blot analysis confirmed that the five piglets contained one disrupted α1,3GT allele.
7. Denning et al. (Nat. Biotechnol 19:559, 2001) describe the deletion of the alpha(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. Eight pregnancies were maintained to term and four PrP-/+ lambs were born.
8. Phelps et al. (Science 299:411-414, 2003) describe production of homozygous α1,3GT knockout pigs. First, the pig α1,3GT gene was used to make heterozygous knockout donor cells, which were then used to clone heterozygous knockout pig. Cells were harvested from the homozygous knockout, and subjected to a second round of targeting, selecting for cells deficient in the Galα(1,3)Gal surface antigen. The double knockout cells were used as donor cells for nuclear transfer to produce homozygous knockout animals.

Applicant asks the Examiner to take notice of the following additional illustrations:

9. Schnieke et al. (Science 278:2130, 1997) report production of human factor IX transgenic sheep. Ovine fibroblasts were transfected with the human factor IX gene, and used as donors for nuclear transfer to enucleated oocytes. Six live transgenic lambs were born, of which three contained the factor IX gene.
10. Cibelli et al. (Science 280:1256, 1998) transfected bovine fibroblasts with a marker gene, which were then fused to enucleated mature oocytes. Out of 28 embryos transferred, three health transgenic calves were isolated.

11. Kuroiwa et al. (Nature Genetics 36:775, 2004) have produced cattle that are homozygous for inactivation of the bovine gene encoding IgM μ-chain (IGHM). Cells were targeted on one allele and used as donors to make heterozygous fetuses. Tissue was harvested, retargeted using *non-isogenic* vectors, and used to make homozygous knockout animals. *Five rounds* of harvesting fetal tissue, genetic modification, and nuclear transfer, produced tissue with this genotype: homozygous inactivation of IGHM, containing a *Cre* transgene, and homozygous inaction of the PRNP gene (responsible for mad cow disease). Nine pregnancies having the five modifications have survived beyond 60 days. Kirin Pharmaceuticals intends to use these animals for producing human IgM antibody for therapy.
12. Ramsoondar et al. (Biol. Reprod. 69:437, 2003) reported the production of pigs containing both a α1,3GT knockout and an α(1,2)fucosyltransferase transgene. Donor fibroblasts *already contained a genetic modification* — the α1,2FT transgene. They were targeted at the α1,3GT locus with *non-isogenic* DNA, producing cells that had two genetic modifications — which were then used successfully for nuclear transfer.
13. Sendai et al. (Transplantation 76:900, 2003) reported production of heterozygous α1,3GT cattle. One fetus was produced from 24 cloned embryos. A fibroblast cell line was established from the fetus for second round targeting, intended for cloning into a homozygous knockout.
14. Kolber-Simonds et al. at Immerge BioTherapeutics (Proc. Natl. Acad. Sci. USA 101:7335, 2004) are *another group* to report production of *homozygous α1,3GT knockout pigs*. Cell lines established from heterozygous knockout cells were selected for spontaneous inactivation of the second allele using antibody staining. They were then used successfully as nuclear donors: 48 transfers resulted in 17 pregnancies, and 4 homozygous α1,3GT knockout piglets.
15. Dor et al. (Transplantation 78:15, 2004) showed that the five α1,3GT knokcout pigs produced by Immerge BioTherapeutics et al. have essentially no expression of Galα(1,3)Gal as determined by IB4 staining (Table 1, Figure 1) and have naturally occurring cytotoxic anti-Galα(1,3)Gal antibody (Figure 3).

The published data support the following conclusions:

- Pigs, cattle and sheep can all be cloned by the Campbell & Wilmut method using genetically altered donor cells to make genetically modified animals.
- Cells can be genetically modified and cloned by nuclear transfer through at least *five cycles*, and still retain their ability to make viable embryos having all the genetic modifications.
- α1,3GT knockouts fetuses have been produced by nuclear transfer in *three different species*.
- Non-isogenic targeting constructs have been used successfully to make α1,3GT knockout pigs and IGHM knockout cattle.
- Homozygous knockouts of any one of several genes can be made without difficulty by harvesting cells from heterozygous animals or fetuses, inactivating the second allele, and then doing a second round of nuclear transfer.
- Homozygous α1,3GT knockout cells for use as nuclear donors can readily be made and identified by several different techniques.
- Two independent groups have successfully made homozygous α1,3GT knockout pigs, which are healthy and essentially free of the Galα(1,3)Gal antigen.

There is no reason to believe that making α1,3GT knockout sheep should be any more difficult to make than α1,3GT knockout pigs. Other than the sheep α1,3GT gene itself, there is nothing critical to the making of α1,3GT knockout sheep that is missing from what was generally known in the art at the time this patent application was filed.

3. Phenotype of α1,3GT Knockouts

Cells from homozygous knockout animals will have cells and tissues lacking the Galα(1,3)Gal xenoantigen.

As described in the specification, the α1,3GT gene is uniquely responsible for forming the Galα(1,3)Gal xenoantigen in non-Catarrhine mammals. An animal that is homozygous for inactivation of the α1,3GT gene would therefore lack the enzyme responsible for making the Galα(1,3)Gal epitope, and would necessarily be deficient in expressing the epitope at the cell surface.

Published data on α1,3GT knockout animals of several species confirm this expectation:

- U.S. Patent 5,849,991 shows that α1,3GT knockout mice essentially lack Galα(1,3)Gal antigen. Peripheral blood monocytes and splenocytes from the homozygous knockouts were analyzed for presence of the Galα(1,3)Gal antigen using the IB4 lectin. Wild-type mice showed high degree of staining, while knockout mice showed minimal staining (Cols. 48-52).
- Phelps et al. (Science 299, 411-414, 2003) show that α1,3GT knockout pigs also essentially lack Galα(1,3)Gal antigen. Cells of these animals are devoid of antibody-detectable Galα(1,3)Gal. See Fig. 1., clones B1-1, B1-2, and B1-4; and Fig. 2.
- Kolber-Simonds et al. (Proc. Natl. Acad. Sci. USA 101:7335, 2004) also show that α1,3GT knockout pigs essentially lack Galα(1,3)Gal antigen. Staining with the IB4 lectin is superimposable in the homozygous knockouts as unstained cells (Fig. 6). The knockout cells were not opsonized for complement lysis by antibody from baboon or human serum, even though normal pig cells were.

The Office Action cites Nebert et al. (Biochem. Pharmacol. 53:249, 1997) and Linder (Lab. Anim. NY 30:34, 2001) as indicating that the phenotype can be altered by such things as genetic background, mutation, site of integration, and genetic modifiers. However, these are things that affect positive expression of a *newly inserted transgene*.

That is *not* the situation here. The α1,3GT gene encodes the α1,3GT enzyme, which in turn builds the Galα(1,3)Gal antigen. Inactivate the gene, and the synthetic pathway is blocked. The particular way by which the gene is knocked out, the genetic back ground, and so on, are all irrelevant, because the gene is still knocked out. *Quod erat demonstrandum.*

4. Purported Lethality of α1,3GT Knockouts

The Office Action states that *contrary to the pig, knockout of α1,3GT gene kills ovine fetuses* (page 17).

This statement is without foundation. The Galα(1,3)Gal antigen has no known biological function that is required for survival. All upper primates lack α1,3GT, and seem to get along quite well without it. Homozygous α1,3GT knockouts have been made in the mouse and the pig without difficulty. There is no evidence of record to indicate that sheep are any different.

Denning et al. (Nat. Biotechnol 19:559, 2001) reported that a few pregnancies of α1,3GT knockout sheep did not reach term. This can be accounted for entirely from the rather low frequency of successful cloning events in mammals. See refs. 5-8 and 12-14 above. Just because an experiment needs to be repeated several times in order to work, it does not mean that undue experimentation is required. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

In summary, the specification fully enables the making of α1,3GT knockout cells and animals by a number of different techniques. The only ingredient needed beyond what was already known is the sheep α1,3GT gene — which is provided in this application for the first time. Using the sequence data or biological deposit in the context of known methodology for nuclear transfer, the skilled reader can make the claimed invention without undue experimentation.

Withdrawal of this rejection is respectfully requested.

Rejection of Claim 16

Claim 16 also stands rejected under 35 USC § 112 ¶ 1 as not being enabled by the specification, because even if ovine tissue devoid of Galα(1,3)Gal determinants could be produced, it would not solve all of the issues that trigger a xenograft response.

Applicant disagrees. The application solves the problem of the Galα(1,3)Gal present on sheep tissue, which would generate hyperacute rejection upon transplantation to a human. The skilled reader would recognize that other issues in transplantation therapy should also be addressed, for example, by the use of immunosuppression and other supportive care that are standard in the transplantation setting. Standard immunosuppressive drugs such as cyclosporin A have a long established track record for overcoming immune rejection of grafted organs (Braun, J. Clin. Apheresis 18:141, 2003; Mueller, Ann. Thorac. Surg. 77:354, 2004), and would be adopted as a matter of course in the use of the claimed invention.

In fact, xenografting has been an established protocol for cardiac valve replacement for almost 40 years: O'Brien et al, Lancet 1:929, 1967. Long-term postoperative survival rates have been between 78% and 94%, depending on what procedure is performed (Stinson et al., J. Thorac. Cardiovasc. Surg. 73:54, 1977; Angell et al., Ann. Thorac. Surg. 28:537, 1979). The success is not impaired by the fact that the value tissue used is taken from animals that normally express Galα(1,3)Gal. The Office Action does not explain why the tissue of this invention would not be effective for therapeutic applications such as cardiac valve therapy.

Furthermore, Costa et al. (FASEB J. 17:109, 2003) reported experiments in which xenograft survival was tested in a model where cartilage was transplanted from transgenic pigs to α1,3GT knockout mice. The pig tissue expressed the transgene α(1,2)fucosyltransferase which reduces but does not eliminate expression of Galα(1,3)Gal. Control pig cartilage grafted into the mice was rejected in several weeks in a cell mediated response. In contrast, the mice receiving the tissue with the transgene showed a markedly reduced anti-pig antibody response and no Galα(1,3)Gal elicited antibody response. There was a mild cellular infiltrate that was confined to the graft periphery, conferring resistance to delayed rejection. In a cardiac transplant model, Chen et al. (C.G. Chen et al. Transplantation 65:832, 1998) showed that α1,2FT transgenic and α1,3GT knockout tissue were both protected against hyperacute rejection.

It can therefore be anticipated that the use of α1,3GT knockout tissue (having an even lower level of Galα(1,3)Gal antigen), in combination with standard immunosuppressive regimens, will enable survival of therapeutically important grafts according to the claimed invention.

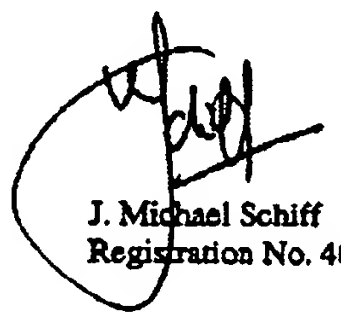
Conclusion

Applicant respectfully requests that all outstanding rejections be reconsidered and withdrawn. The application is believed to be in condition for allowance, and a prompt Notice of Allowance is requested.

In the event that the Examiner determines that there are other matters to be addressed, applicant hereby requests an interview by telephone.

Should the Patent Office determine that an extension of time or any other relief is required for further consideration of this application, applicant hereby petitions for such relief, and authorizes the Commissioner to charge the cost of such petitions and other fees due in connection with the filing of these papers to Deposit Account No. 07-1139, referencing the docket number indicated above.

Respectfully submitted,



J. Michael Schiff
Registration No. 40,253

GERON CORPORATION
230 Constitution Drive
Menlo Park, CA 94025
Telephone: (650) 473-7715
Fax: (650) 473-8654

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